An optimised Cu(0)-RDRP approach for the synthesis of lipidated oligomeric vinyl azlactone: toward a versatile antimicrobial materials screening platform.

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METHODS

Polymerisation of O(VDM) using DBib and BMPD.

VDM (0.4 mL, 2.99 mmol, 10 eq.), DMSO (1.0 mL), DBib (0.0948 mL, 0.299 mmol, 1 eq.), Me_6TREN (0.0463 mL, 0.173 mmol, 0.58 eq.), $CuBr_2$ (0.0200 g, 0.0897 mmol, 0.30 eq.), were charged to a polymerisation flask with a magnetic stir bar and fitted with a rubber septum and the mixture degassed via nitrogen sparging for 15 min after which pre-activated copper wire was carefully added under a nitrogen blanket. The polymerisation flask was then resealed and deoxygenated for a further five minutes. The solution was allowed to polymerise for 3 hours, after which samples were taken for ^{1}H NMR spectroscopy and GPC analyses.

VDM (0.2 mL, 1.50 mmol, 10 eq.), DMSO (1.0 mL), BMPD (0.0905 g, 0.149 mmol, 1 eq.), Me_6TREN (0.0231 mL, 0.0867 mmol, 0.58 eq.), $CuBr_2$ (0.0100 g, 0.0448 mmol, 0.30 eq.), were charged to a polymerisation flask with a magnetic stir bar and fitted with a rubber septum and the mixture degassed via nitrogen sparging for 15 min after which pre-activated copper wire was carefully added under a nitrogen blanket. The polymerisation flask was then resealed and deoxygenated for a further five minutes. The solution was allowed to polymerise for 3 hours, after which samples were taken for ^{1}H NMR spectroscopy and GPC analyses.

The sample for ^{1}H NMR analysis was diluted with $CDCl_3$, while the sample for GPC analysis was diluted with DMAc and passed over a neutral aluminium oxide column to remove any metal salts.

Ring opening of O(VDM) with 2-(2-aminoethyl)-1,3-di-Boc-guanidine.

Crude C_{12}-O(VDM) solution (0.388 mL; containing 0.828 mmol of the azlactone functionality) was added to a new vial and diluted with DMSO (0.431 mL). 2-(2-Aminoethyl)-1,3-di-Boc-guanidine (275.54 mg, 0.911 mmol) and TEA (0.116 mL) in DMSO (0.862 mL) and DMF (1 mL) were added to the oligomer solution. The solution was allowed to stir at room temperature for three days.

Crude $2C_{12}$-O(VDM) solution (0.225 mL; containing 0.562 mmol of the azlactone functionality) was added to a new vial and diluted with DMSO (0.251 mL). 2-(2-Aminoethyl)-1,3-di-Boc-guanidine (186.83 mg, 0.618 mmol) and TEA (0.078 mL) in DMSO (0.501 mL) and DMF (1 mL) were added to the oligomer solution. The solution was allowed to stir at room temperature for three days.

Ring opening of O(VDM) with 1-(3-aminopropyl)imidazole.

Crude C_{12}-O(VDM) solution (0.279 mL; containing 0.695 mmol of the azlactone functionality) was added to a new vial and diluted with DMSO (0.310 mL). 1-(3-Aminopropyl)imidazole (95.68 mg, 0.764 mmol) and TEA (0.097 mL) in DMSO (0.620 mL) were added to the oligomer solution. The solution was allowed to stir at room temperature for three days.

Crude $2C_{12}$-O(VDM) solution (0.225 mL; containing 0.562 mmol of the azlactone functionality) was added to a new vial and diluted with DMSO (0.251 mL). 1-(3-Aminopropyl)imidazole (77.34 mg, 0.618 mmol) and TEA (0.078 mL) in DMSO (0.501 mL) were added to the oligomer solution. The solution allowed to stir at room temperature for three days.

Ring opening of O(VDM) with *N*-Boc-ethylenediamine.

Crude C_{12}-O(VDM) solution (0.310 mL; containing 0.695 mmol of the azlactone functionality) was added to a new vial and diluted with DMSO (0.310 mL). BEDA (122.47 mg, 0.764 mmol) and TEA (0.097 mL) in DMSO (0.620 mL) were added to the oligomer solution. The solution was allowed to stir at room temperature for three days.

Crude $2C_{12}$-O(VDM) solution (0.225 mL; containing 0.562 mmol of the azlactone functionality) was added to a new vial and diluted with DMSO (0.251 mL). BEDA (98.99 mg, 0.618 mmol) and TEA (0.078 mL) in DMSO (0.620 mL) were added to the oligomer solution. The solution was allowed to stir at room temperature for three days.

Ring opening of O(VDM) with *N*,*N*-dimethylethylenediamine.

Crude C_{12}-O(VDM) solution (0.388 mL; containing 0.828 mmol of the azlactone functionality) was added to a new vial and diluted with DMSO (0.431 mL). DMEN (80.33 mg, 0.911 mmol) and TEA (0.116 mL) in DMSO (0.862 mL) were added to the oligomer solution. The solution was allowed to stir at room temperature for three days.

Crude $2C_{12}$-O(VDM) solution (0.225 mL; containing 0.562 mmol of the azlactone functionality) was added to a new vial and diluted with DMSO (0.251 mL). DMEN (54.47 mg, 0.618 mmol) and TEA (0.078 mL) in DMSO (0.501 mL) were added to the oligomer solution. The solution was allowed to stir at room temperature for three days.

Deprotection of oligomers ring opened with 2-(2-aminoethyl)-1,3-di-Boc-guanidine.

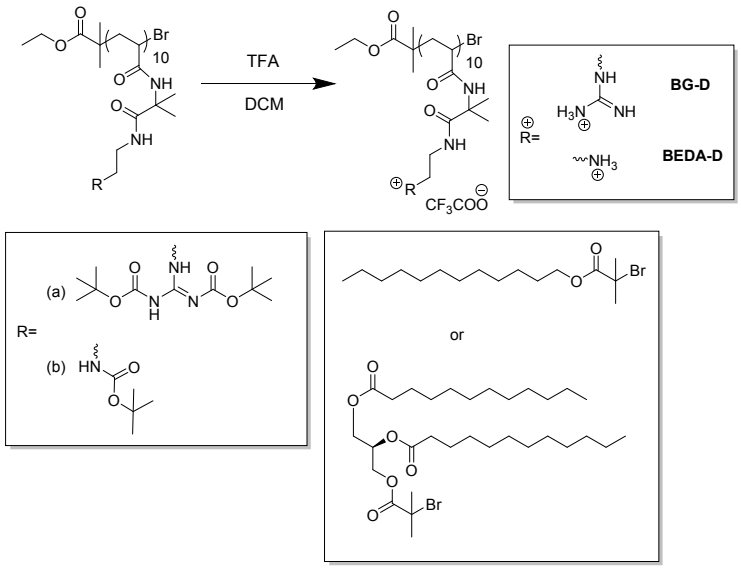
A representative procedure is detailed below.

C_2-O(BG) (100 mg) was dissolved in DCM (1.0 mL), in which TFA (1.0 mL) was added and allowed to react overnight. The resulting deprotected oligomer solution was then evaporated to dryness under a stream of air, 5.0 mL of acetone was added and evaporated to dryness under a stream of air, and this was repeated three times. This procedure was then repeated for the C_{12}- and $2C_{12}$-terminated oligomers. Complete deprotection was confirmed by ^{1}H NMR spectroscopy.

Deprotection of oligomers ring opened with N-Boc-ethylenediamine.

A representative procedure is detailed below.

C_2-O(BEDA) (100 mg) was dissolved in DCM (1.0 mL), in which TFA (1.0 mL) was added and allowed to react overnight. The resulting deprotected oligomer solution was then evaporated to dryness under a stream of air, 5.0 mL of acetone was added and evaporated to dryness under a stream of air, and this was repeated three times. This procedure was then repeated for the C_{12}- and $2C_{12}$-terminated oligomers. Complete deprotection was confirmed by ^{1}H NMR spectroscopy.



Scheme SI 1 Representative scheme of the deprotection of Boc-groups utilising trifluoroacetic acid.

^{1}H NMR peak integration analysis for calculation of M_n and DP_n.

The DP_n of the DMEN-containing oligomers was calculated using the ratio of an integral associated with protons in the repeat unit to an integral associated with protons in the end-group. The DP_n of the C_2-terminated DMEN-containing oligomer (see Figure SI 10A) was determined by using the ratio of the peak 'g' at 3.37 ppm (corresponding to the –NH-C**H$_2$**-CH$_2$-N- protons) to peak 'b' at 4.17 ppm (corresponding to CH$_3$-C**H$_2$**-O protons in the alkyl tail). This could be summarised using the formula:

$$DP_n\left(C_2(O(DMEN))\right) = \frac{I_{3.37}}{I_{4.17}}$$

This calculation was then repeated for C_{12}- and $2C_{12}$-terminated DMEN-containing oligomers (see Figure SI 12A and Figure SI 14A, respectively). The DP_n was determined by using the ratio of the

peak at ~3.37 ppm (corresponding to the –NH-C**H₂**-CH**₂**-N- protons) to peak ‘a’ at 0.87 ppm (corresponding to the terminal methyl group in the alkyl tail). This could be summarised using the formulas below for C_{12}- and $2C_{12}$-terminated DMEN-containing oligomers, respectively.

$$DP_n\left(C_{12}(O(DMEN))\right) = \frac{3 \times I_{3.35}}{2 \times I_{0.87}}$$

$$DP_n\left(2C_{12}(O(DMEN))\right) = \frac{6 \times I_{3.37}}{2 \times I_{0.87}}$$

The DP_n of the BEDA-containing oligomers was calculated using the ratio of an integral associated with protons in the repeat unit to an integral associated with protons in the end-group. The DP_n of the C_2-terminated BEDA-containing oligomer (see Figure SI 10B) was determined by using the ratio of the peak ‘g, h’ from 3.00 to 3.50 ppm (corresponding to the –NH-C**H₂**-C**H₂**-NH- protons) to peak ‘b’ at 4.10 ppm (corresponding to CH_3-C**H₂**-O protons in the alkyl tail). This could be summarised using the formula:

$$DP_n\left(C_2(O(BEDA))\right) = \frac{2 \times I_{3.28}}{4 \times I_{4.10}}$$

This calculation was then repeated for C_{12}- and $2C_{12}$-terminated BEDA-containing oligomers (see Figure SI 12B and Figure SI 14B). The DP_n was determined by using the ratio of the peak from 3.00 to 3.60 ppm (corresponding to the –NH-C**H₂**-C**H₂**-NH- protons) to peak ‘a’ at 0.88 ppm (corresponding to the terminal methyl group in the alkyl tail). This could be summarised using the formulas below for C_{12}- and $2C_{12}$-terminated BEDA-containing oligomers, respectively.

$$DP_n\left(C_{12}(O(BEDA))\right) = \frac{3 \times I_{3.28}}{4 \times I_{0.88}}$$

$$DP_n\left(2C_{12}(O(BEDA))\right) = \frac{6 \times I_{3.27}}{4 \times I_{0.88}}$$

The DP_n of the C_{12} and $2C_{12}$-terminated Boc-guanidine-containing oligomers was calculated using the ratio of an integral associated with protons in the repeat unit to an integral associated in the end-group (see Figure SI 12C and Figure SI 14C). The DP_n was determined by using the ratio of the peak from 3.15 to 3.90 ppm (corresponding to the –NH-C**H₂**-C**H₂**-NH- protons) to peak ‘a’ at 0.88 ppm (corresponding to the terminal methyl group in the alkyl tail). This could be summarised using the formulas below for C_{12}- and $2C_{12}$-terminated DMEN-containing oligomers, respectively.

$$DP_n(C_{12}(O(BG))) = \frac{3 \times I_{3.15}}{4 \times I_{0.88}}$$

$$DP_n(2C_{12}(O(BG))) = \frac{6 \times I_{3.15}}{4 \times I_{0.87}}$$

The DP_n of the C_{12} and $2C_{12}$-terminated imidazole-containing oligomers was calculated using the ratio of an integral associated with protons in the end-group (see Figure SI 12D and Figure SI 14D). The DP_n was determined by using the ratio of the peak at ~3.92 ppm (corresponding to the -NH-CH_2-CH_2-C**H_2**-protons) to peak 'a' at 0.84 ppm (corresponding to the terminal methyl group in the alkyl tail). This could be summarised using the formulas below for C_{12}- and $2C_{12}$-terminated DMEN-containing oligomers, respectively.

$$DP_n(C_{12}(O(Imid))) = \frac{3 \times I_{3.92}}{2 \times I_{0.84}}$$

$$DP_n(2C_{12}(O(Imid))) = \frac{6 \times I_{3.91}}{2 \times I_{0.84}}$$

The number average molecular weight (M_n) was calculated by using the DP_n calculated previously for the VDM block, the DP_n calculated for the block length of the ring opening reactant and the molecular weight of the initiator. See below for a representative equation:

$$M_n = DP_n \times MW(VDM) + DP \times MW(Ring\ opening\ reactant) + MW(Initiator)$$

The theoretical number average molecular weight ($M_{n,th}$) was calculated by using the DP_n calculated previously for the VDM block, the DP_n calculated for the block length of the ring opening reactant and the molecular weight of the initiator. See below for a representative equation:

$$M_{n,th} = Conv.(\%) \times \frac{[M]_0}{[I]_0} \times MW(VDM) + DP \times MW(Ring\ opening\ reactant) + MW(Initiator)$$

Compound Preparation and Storage

Stock solutions were prepared by solubilising the oligomers in 100% DMSO and then diluting the solutions with water to 20% DMSO and a final stock concentration of 10.24 mg/mL. After solubilising the polymers, they were stored at 4 °C.

The highest tested concentration for each compound in the bacterial and fungal minimum inhibitory concentration (MIC) assays was 512 μg/mL. As the maximum DMSO in the haemolysis and human cell viability assays is constrained to 0.5%, the highest concentration reported is 256 μg/mL.

Bacterial Broth Microdilution Assay.

MICs were determined as the lowest concentration at which no visible growth could be detected. Also, optical density was read at 600 nm (OD_{600}) using a Tecan M1000 Pro Spectrophotometer to calculate growth inhibition (%):

$$Growth\ inhibition\ (\%) = (1 - \frac{OD_{600\ sample} - OD_{600\ median\ growth\ control}}{OD_{600\ median\ negative\ control} - OD_{600\ median\ growth\ control}}) \times 100$$

$OD_{600\ sample}$ is the optical density when the oligomer is added to the bacterial cells. $OD_{600\ median\ growth\ control}$ is the optical density when the control antibiotic was added to the bacterial cells. $OD_{600\ median\ negative\ control}$ is the optical density of untreated bacterial cells.

Cell Viability Analysis

The data were analysed using Microsoft Excel and GraphPad Prism software. Cytotoxicity or cell viability were calculated using the following equation:

$$Cell\ viability\ (\%) = \frac{FI_{Sample} - FI_{Negative}}{FI_{Untreated} - FI_{Negative}} \times 100$$

Using nonlinear regression analysis of log(concentration) vs normalised cell viability, using variable fitting, CC_{50} (concentration at 50% cell viability) were calculated. Any value with > indicate sample with no inhibition or CC_{50} above the maximum tested concentration. FI_{Sample} is the fluorescence intensity when the oligomer is added to the cells. $FI_{Negative}$ is the fluorescence intensity of the media, and $FI_{Untreated}$ is the fluorescence intensity of untreated cells.

Haemolysis Analysis

The data was analysed using Microsoft Excel and GraphPad Prism software. Percent haemolysis were calculated using the following equation:

$$Haemolysis\ (\%) = \frac{Abs_{Sample} - Abs_{Negative}}{Abs_{Positive} - Abs_{Negative}} \times 100$$

Using nonlinear regression analysis of log(concentration) vs normalised haemolysis, using variable fitting, HC_{10} and HC_{50} (concentration at 10 and 50% haemolysis, respectively) were calculated. In addition, the maximum percentage of haemolysis is reported. Any value with > indicate samples with no haemolytic activity or a HC_{10} or HC_{50} above the maximum tested concentration.

Permeabilisation of *S. aureus* membrane Analysis

The maximal fluorescence values were converted to percentage of PI binding using the equation:

$$\%\ PI\ binding = \frac{F_{treatment} - F_{PBS}}{F_{melittin} - F_{PBS}} \times 100$$

where $F_{treatment}$ is the maximum observed fluorescence at a given treatment concentration, F_{PBS} is the maximum fluorescence of cells with PI and PBS, and $F_{melittin}$ is the maximum fluorescence of melittin-treated cells.

RESULTS

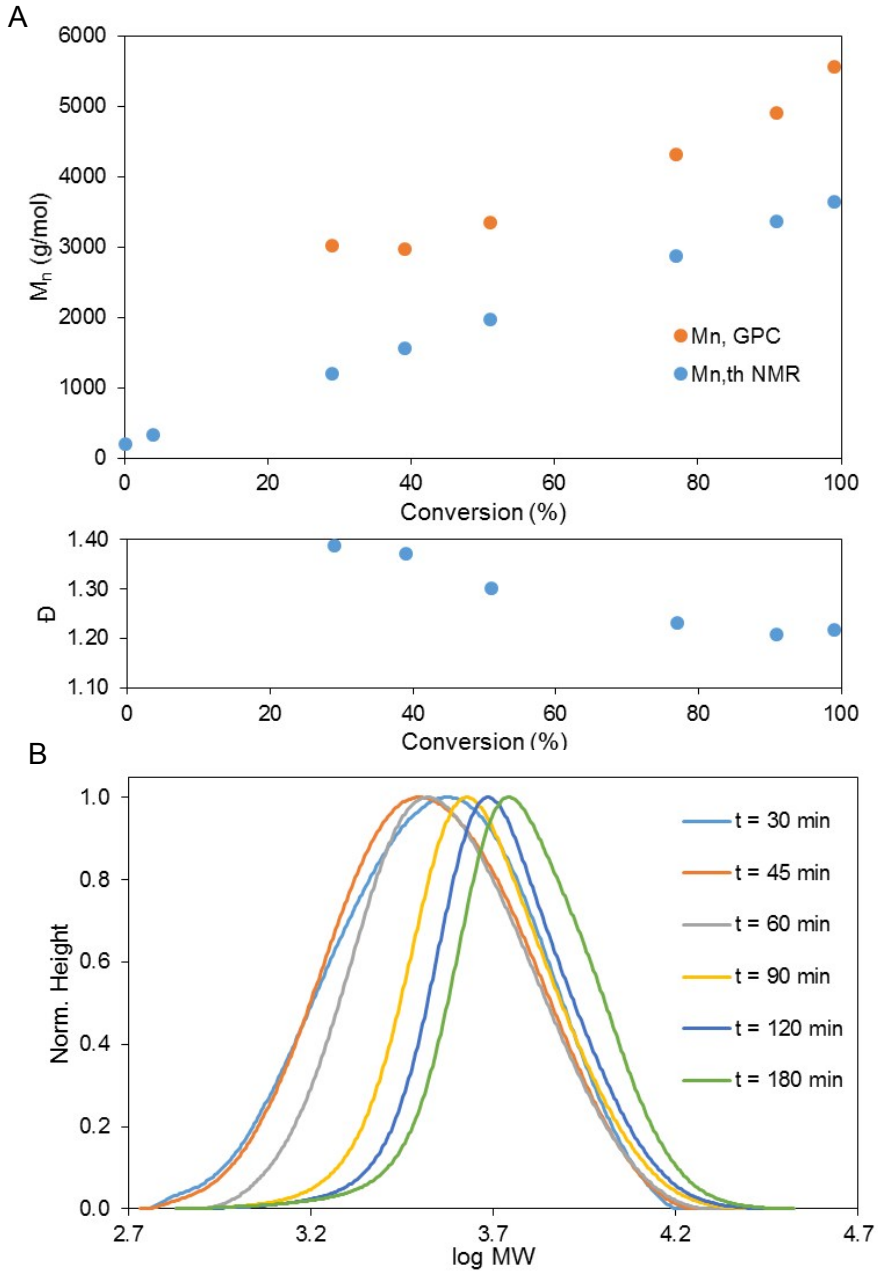


Figure SI 1 Evolution of number average molar mass (M_n), dispersity (Đ), and molar mass distribution as a function of conversion. Reaction conditions: Table 1 Entry 2 ($[M]_0$ = 2.49 M, $[I]_0$ = 0.0997 M, $[Me_6TREN]_0$ = 0.0997 M, $[CuBr_2]_0$ = 0.00498 M) (25/1/1/0.05) in DMSO at 25 °C. A) M_n and Đ vs conversion plot. M_n values (orange points) were calculated from GPC. M_n values (blue points) were derived from ^{1}H NMR conversion calculations. B) Molar mass distributions over the time of the kinetic study. Data corresponds to the final polymer detailed in Table 1 Entry 2.

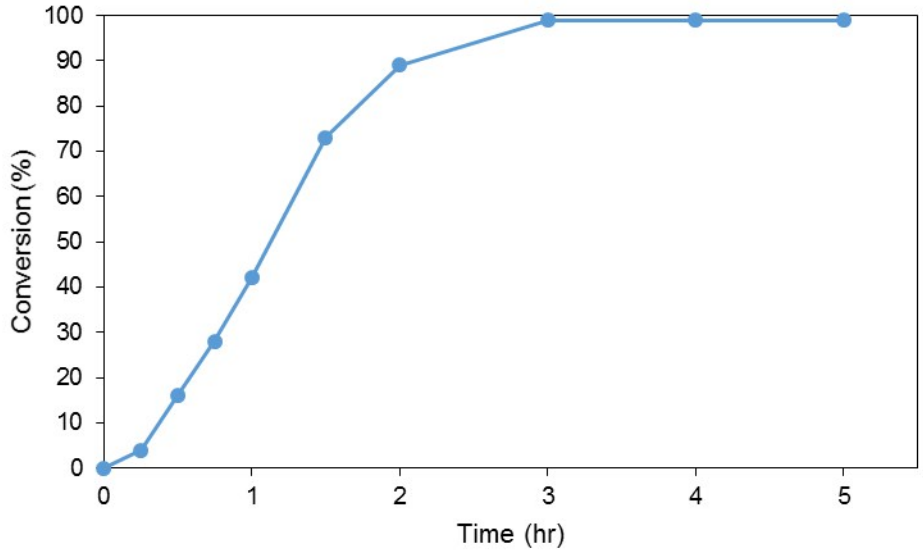


Figure SI 2 Conversion (%) profile for Cu(0)-mediated polymerisation of VDM in DMSO at 25 °C: ([M] = 2.49 M, [I] = 0.0997 M, [Me_6TREN] = 0.0997 M, [$CuBr_2$] = 0.00498 M) (25/1/1/0.05) (see Table 1 Entry 2).

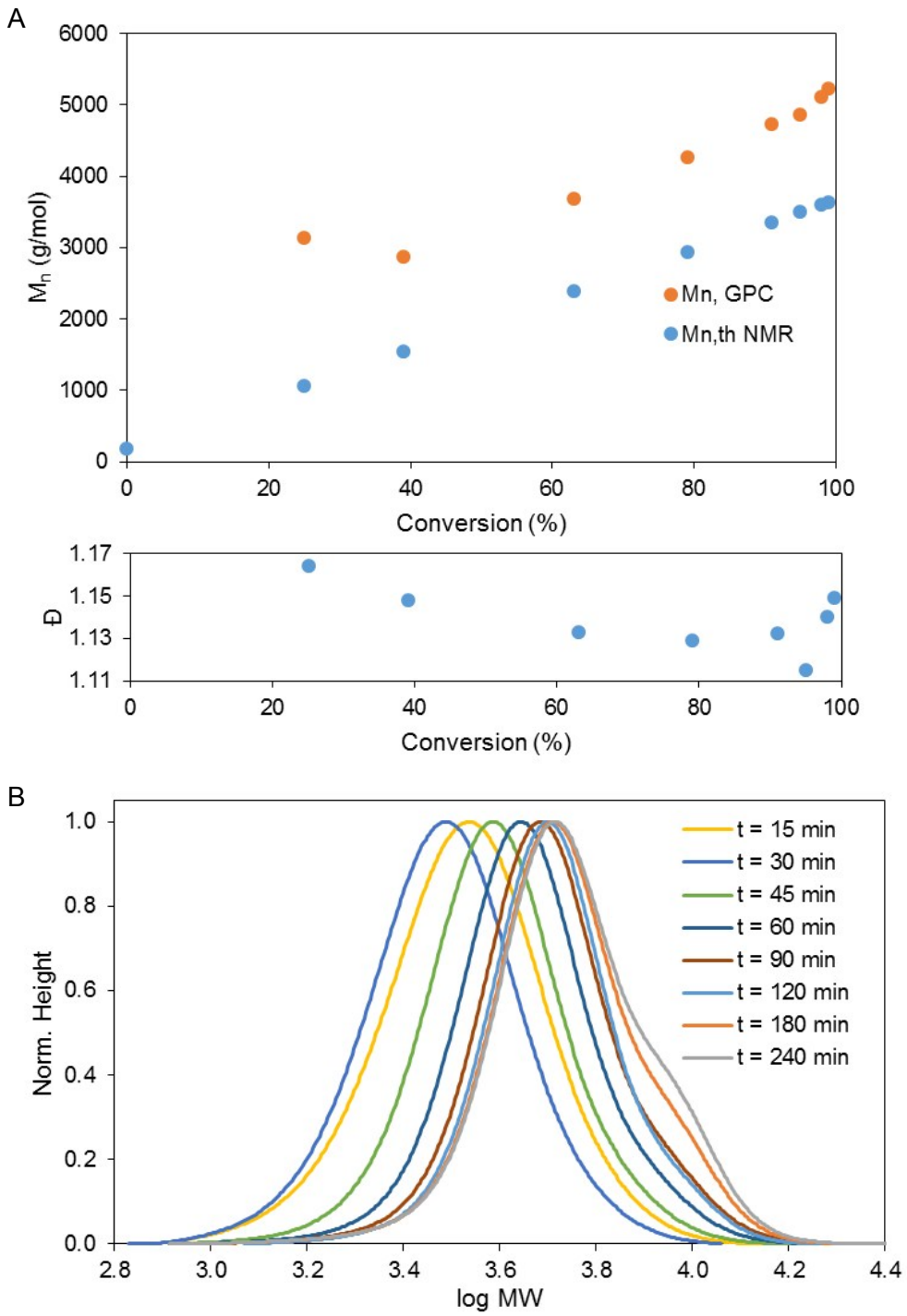


Figure SI 3 Evolution of number average molar mass (M_n), dispersity (Đ), and molar mass distribution as a function of conversion. Reaction conditions: ($[M]_0$ = 2.49 M, $[I]_0$= 0.0997 M, $[Me_6TREN]_0$ = 0.0578 M, $[CuBr_2]_0$ = 0.00498 M) (25/1/0.58/0.05) in DMSO at 25 °C. A) M_n and Đ vs conversion plot. M_n values (orange points) were calculated from GPC. M_n values (blue points) were derived from ^{1}H NMR conversion calculations. B) Molar mass distributions over the time of the kinetic study. Data corresponds to the final polymer detailed in Table 1 Entry 3.

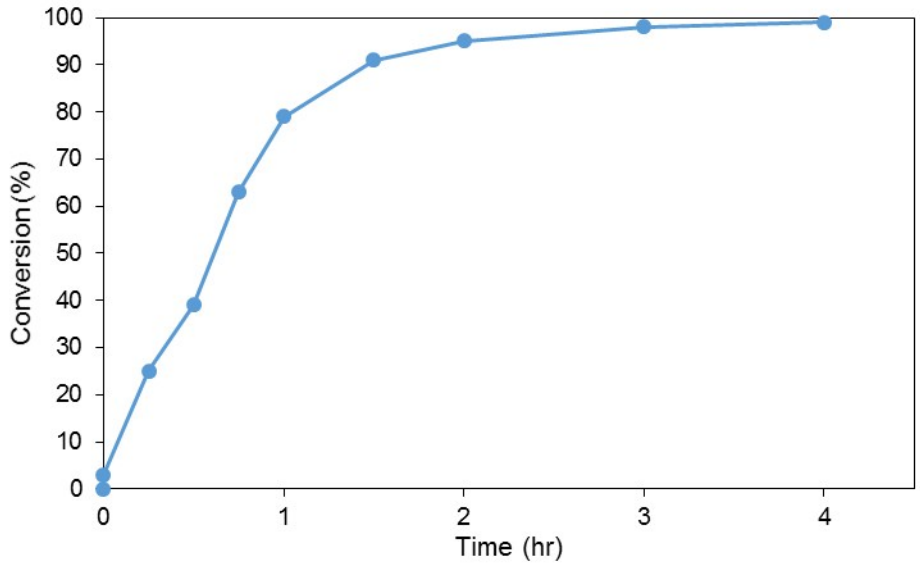


Figure SI 4 Conversion (%) profile for Cu(0)-mediated polymerisation of VDM in DMSO at 25 °C: ([M] = 2.49 M, [I] = 0.0997 M, [Me_6TREN] = 0.0578 M, [$CuBr_2$] = 0.00498 M) (25/1/0.58/0.05) (see Table 1 Entry 3).

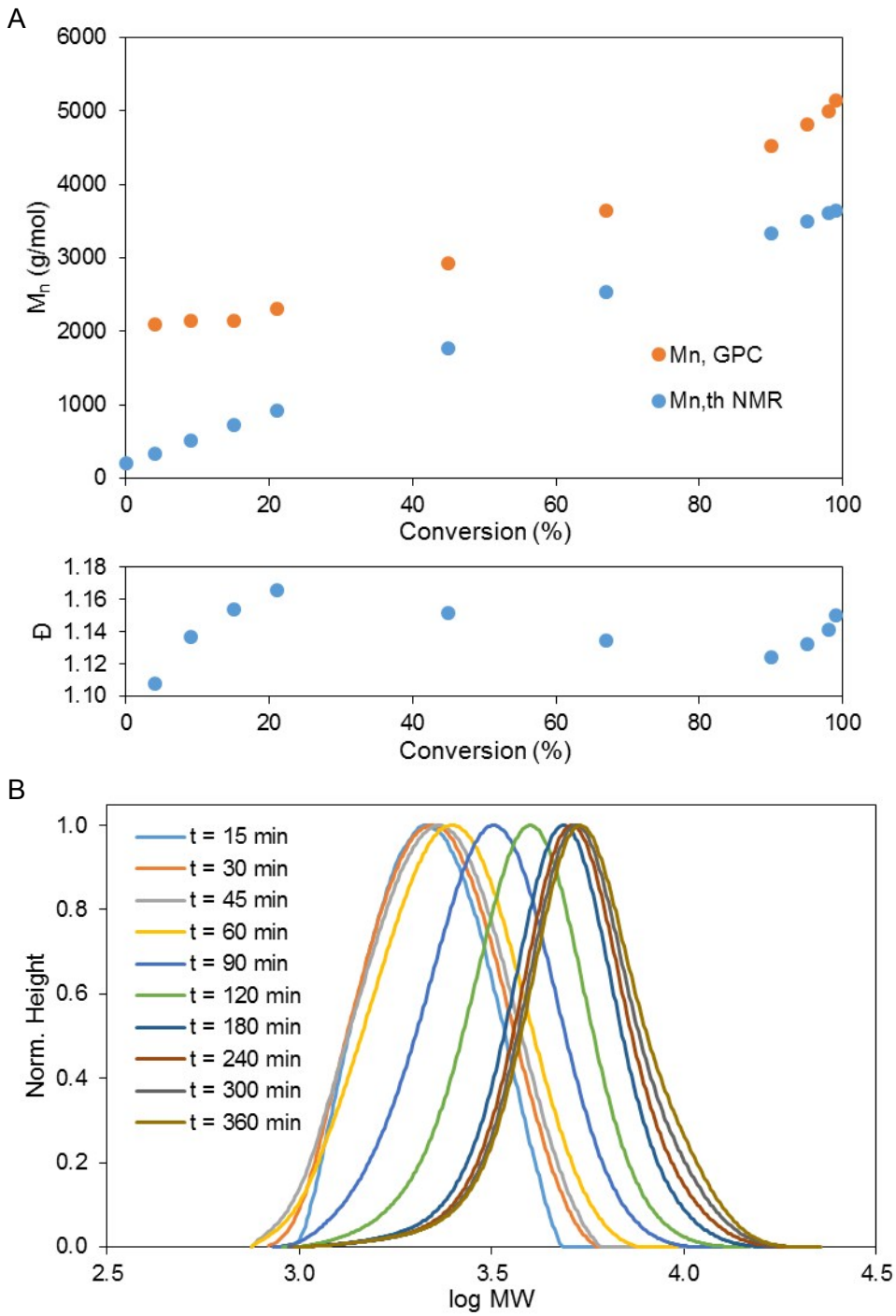


Figure SI 5 Evolution of number average molar mass (M_n), dispersity (Đ), and molar mass distribution as a function of conversion. Reaction conditions: ($[M]_0$ = 2.49 M, $[I]_0$ = 0.0997 M, $[Me_6TREN]_0$ = 0.0578 M, $[CuBr_2]_0$ = 0.00997 M) (25/1/0.58/0.10) in DMSO at 25 °C. A) M_n and Đ vs conversion plot. M_n values (orange points) were calculated from GPC. M_n values (blue points) were derived from ^{1}H NMR conversion calculations. B) Molar mass distributions over the time of the kinetic study. Data corresponds to the final polymer detailed in Table 1 Entry 4.

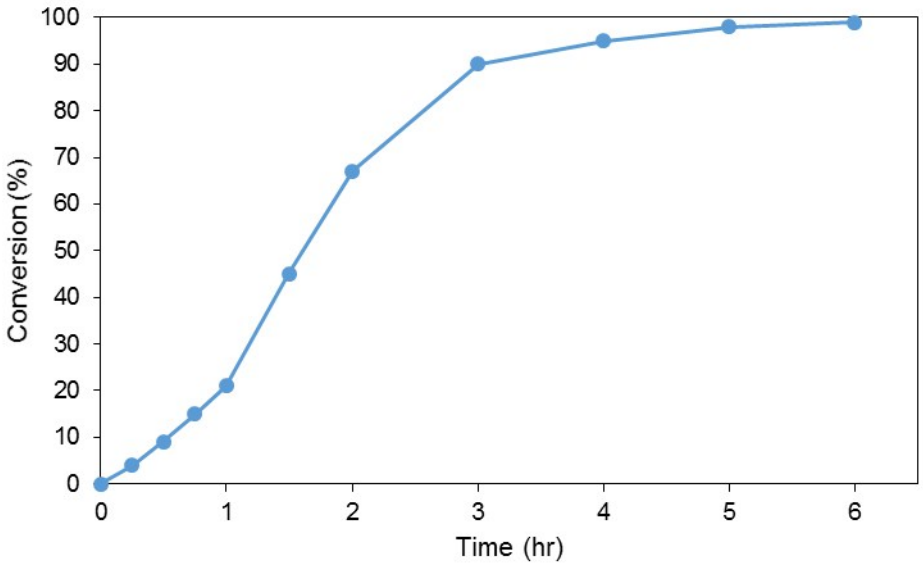


Figure SI 6 Conversion (%) profile for Cu(0)-mediated polymerisation of VDM in DMSO at 25 °C: ([M] = 2.49 M, [I] = 0.0997 M, [Me_6TREN] = 0.0578 M, [$CuBr_2$] = 0.00997 M) (25/1/0.58/0.10) (see Table 1 Entry 4).

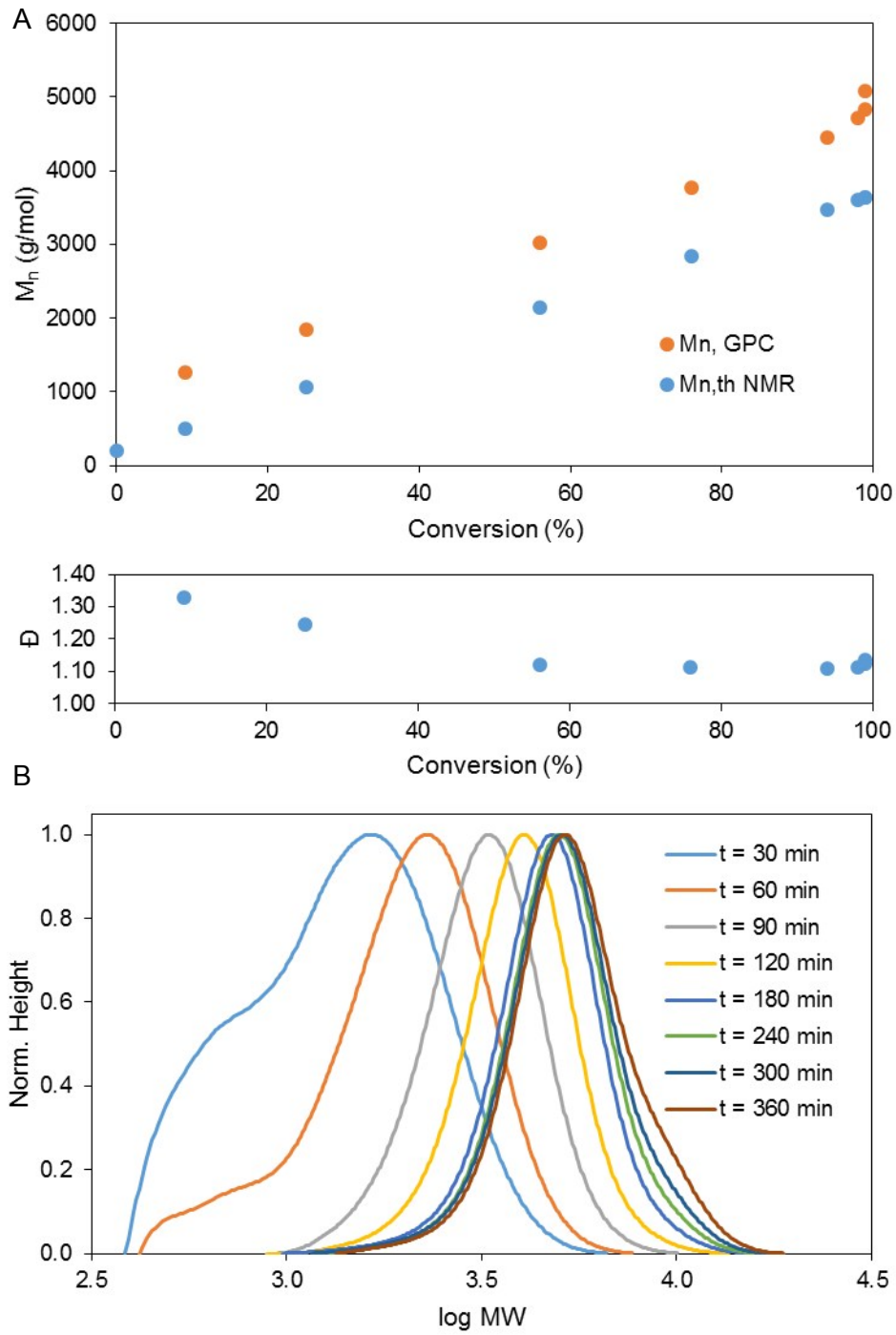


Figure SI 7 Evolution of number average molar mass (M_n), dispersity (Đ), and molar mass distribution as a function of conversion. Reaction conditions: ($[M]_0$ = 2.49 M, $[I]_0$ = 0.0997 M, $[Me_6TREN]_0$ = 0.0578 M, $[CuBr_2]_0$ = 0.0149 M) (25/1/0.58/0.15) in DMSO at 25 °C. A) M_n and Đ vs conversion plot. M_n values (orange points) were calculated from GPC. M_n values (blue points) were derived from ^{1}H NMR conversion calculations. B) Molar mass distributions over the time of the kinetic study. Data corresponds to the final polymer detailed in Table 1 Entry 5.

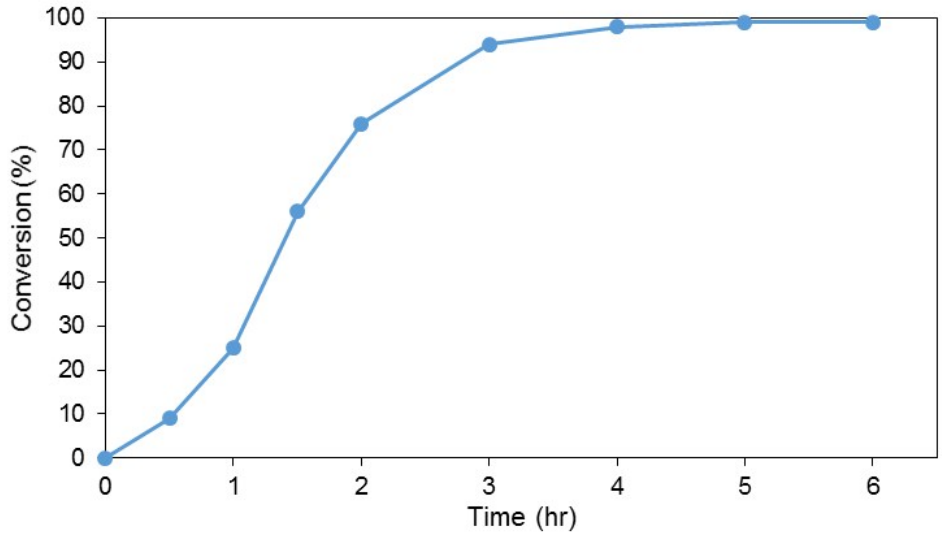


Figure SI 8 Conversion (%) profile for Cu(0)-mediated polymerisation of VDM in DMSO at 25 °C: ([M] = 2.49 M, [I] = 0.0997 M, [Me_6TREN] = 0.0578 M, [$CuBr_2$] = 0.0149 M) (25/1/0.58/0.15) (see Table 1 Entry 5).

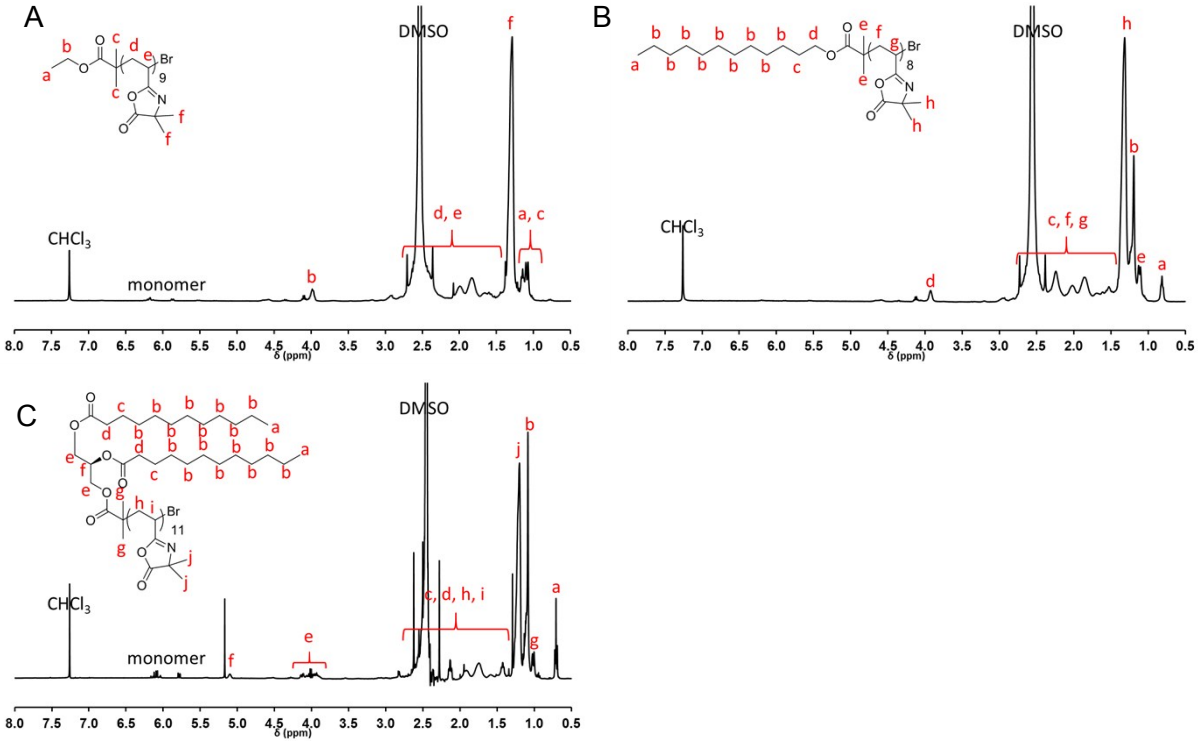


Figure SI 9 ^{1}H NMR spectra of precursor O(VDM) oligomers: A) C_2-O(VDM); C_{12}-O(VDM); and C) $2C_{12}$-O(VDM). ^{1}H NMR conducted in $CDCl_3$.

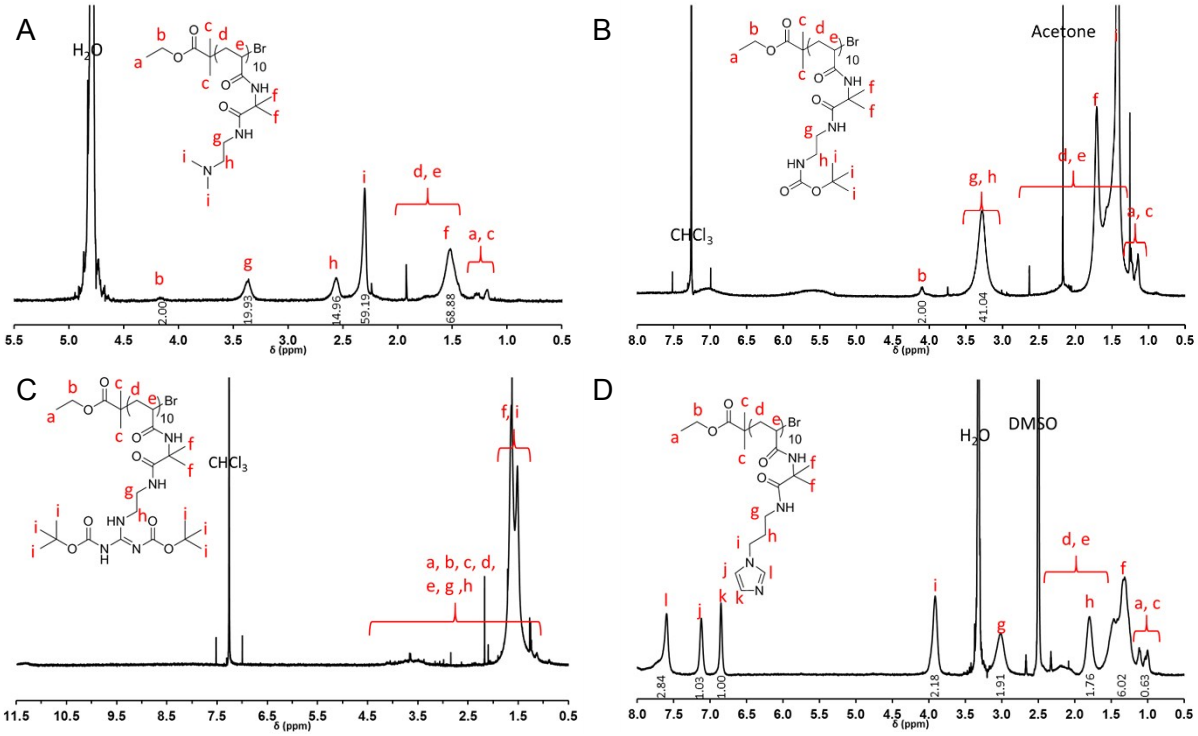


Figure SI 10 ^1H NMR spectra of C_2-O(VDM) ring-opened utilising A) DMEN; B) BEDA; C) 2-(2-aminoethyl)-1,3-di-Boc-guanidine; and D) 1-(3-aminopropyl)imidazole. ^1H NMR was conducted in D_2O for A, $CDCl_3$ for B and C, and DMSO-d_6 for D.

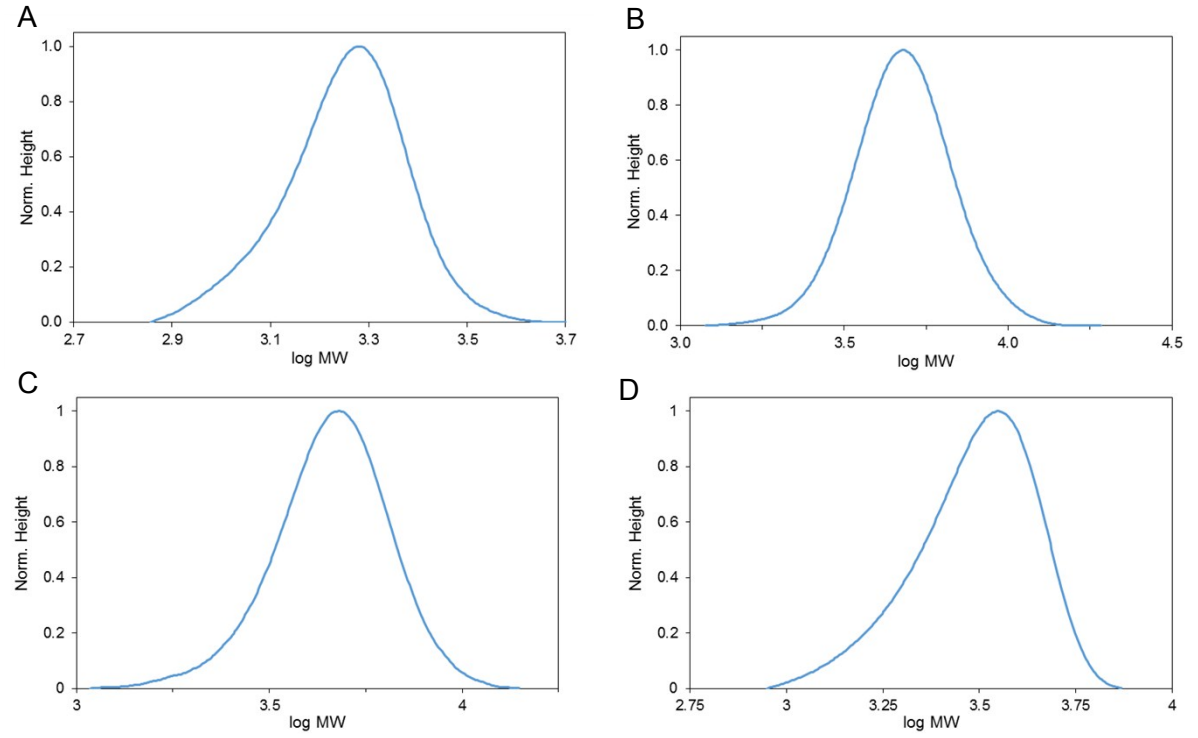


Figure SI 11 Molecular weight distribution of C_2-O(VDM) ring-opened utilising A) DMEN; B) BEDA; C) 2-(2-aminoethyl)-1,3-di-Boc-guanidine; and D) 1-(3-aminopropyl)imidazole. GPC was conducted in DMAc. TEA was added into A.

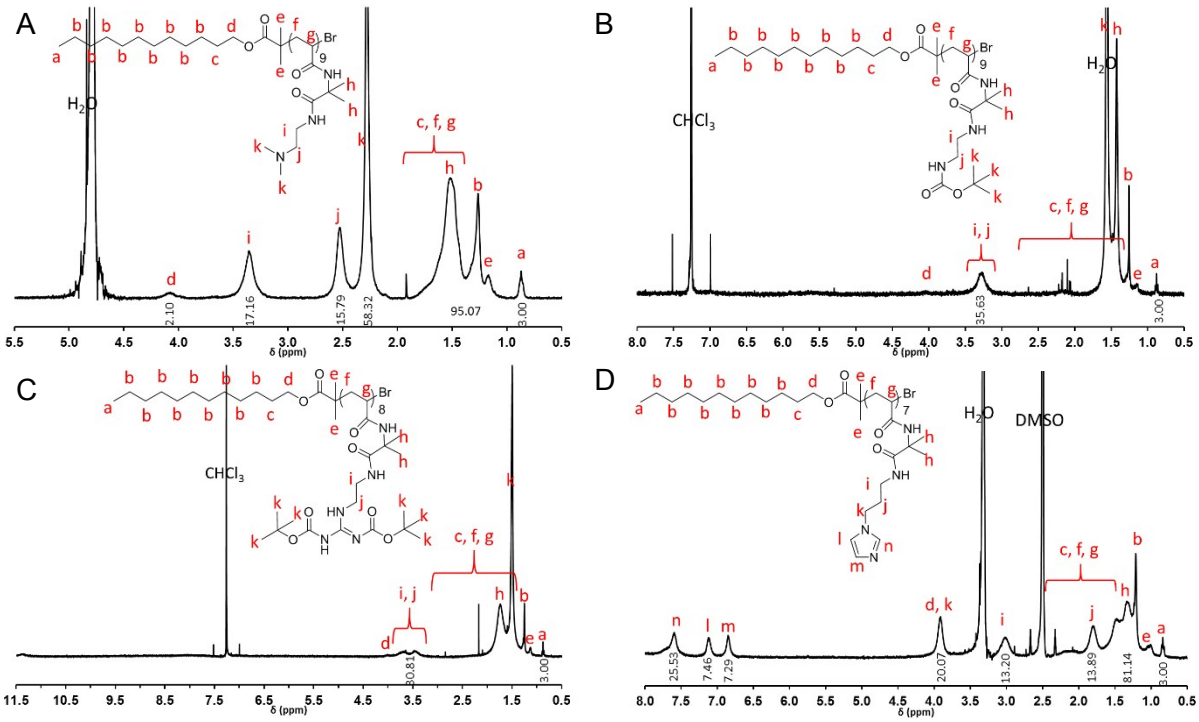


Figure SI 12 ^{1}H NMR spectra of C_{12}-O(VDM) ring-opened utilising A) DMEN; B) BEDA; C) 2-(2-aminoethyl)-1,3-di-Boc-guanidine; and D) 1-(3-aminopropyl)imidazole. ^{1}H NMR was conducted in D_2O for A, $CDCl_3$ for B and C, and DMSO-d_6 for D.

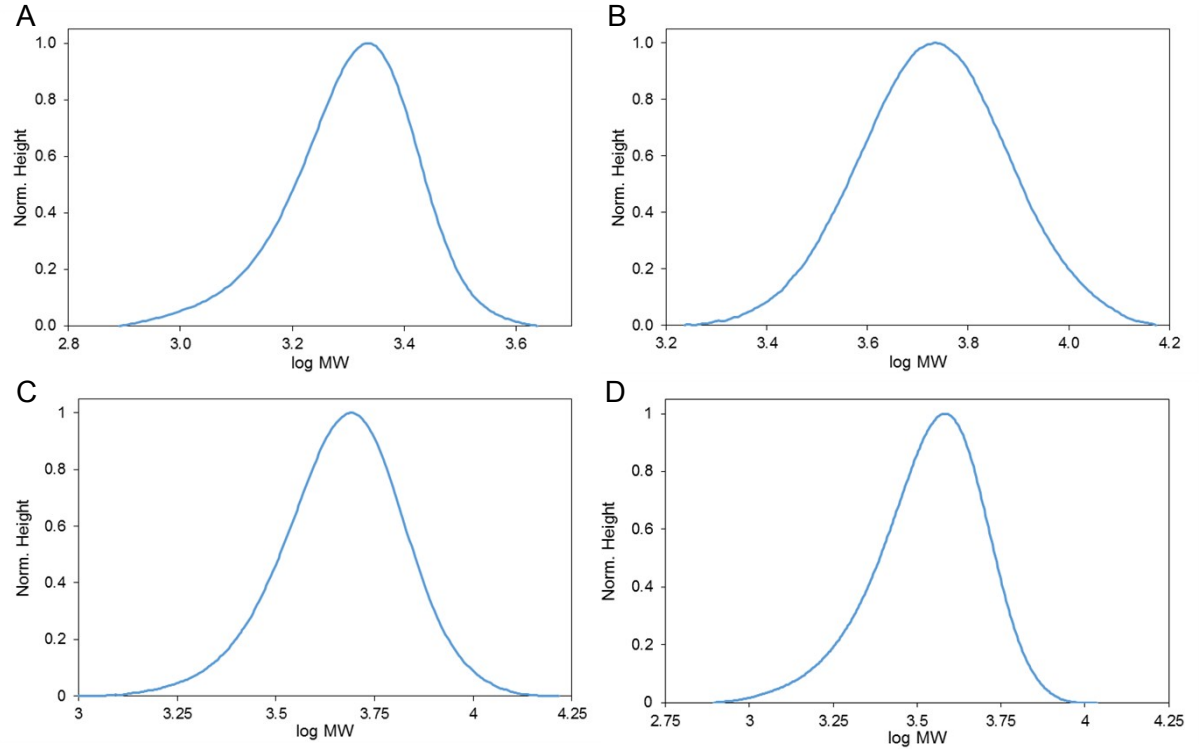


Figure SI 13 Molecular weight distribution of C_{12}-O(VDM) ring-opened utilising A) DMEN; B) BEDA; C) 2-(2-aminoethyl)-1,3-di-Boc-guanidine; and D) 1-(3-aminopropyl)imidazole. GPC was conducted in DMAc. TEA was added into A.

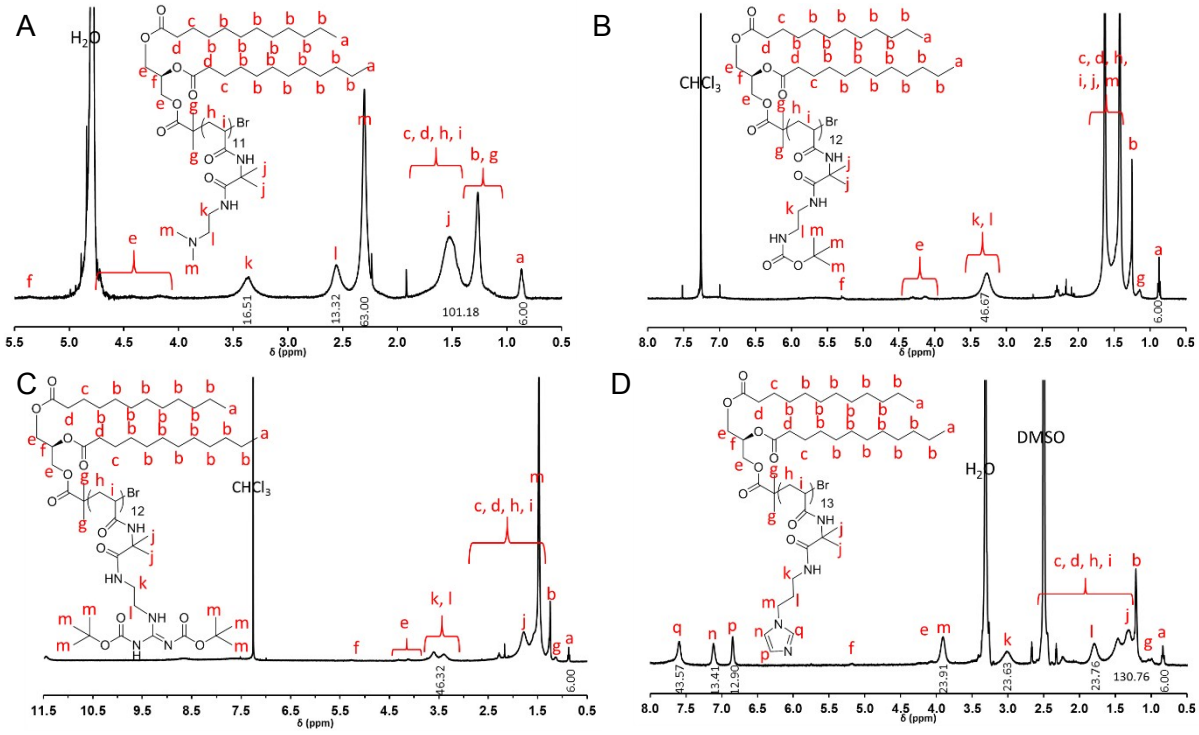


Figure SI 14 ^{1}H NMR spectra of 2C$_{12}$-O(VDM) ring-opened utilising A) DMEN; B) BEDA; C) 2-(2-aminoethyl)-1,3-di-Boc-guanidine; and D) 1-(3-aminopropyl)imidazole. ^{1}H NMR was conducted in D_2O for A, $CDCl_3$ for B and C, and DMSO-d_6 for D.

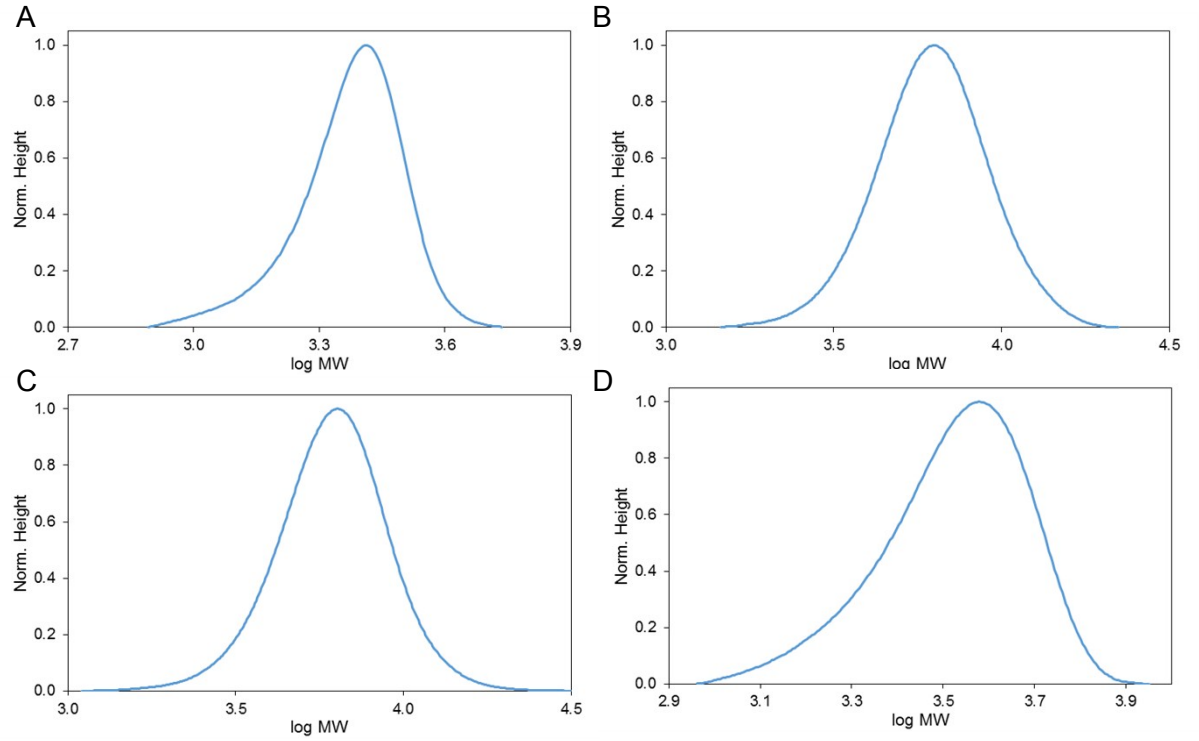


Figure SI 15 Molecular weight distribution of 2C$_{12}$-O(VDM) ring-opened utilising A) DMEN; B) BEDA; C) 2-(2-aminoethyl)-1,3-di-Boc-guanidine; and D) 1-(3-aminopropyl)imidazole. GPC was conducted in DMAc. TEA was added into A.

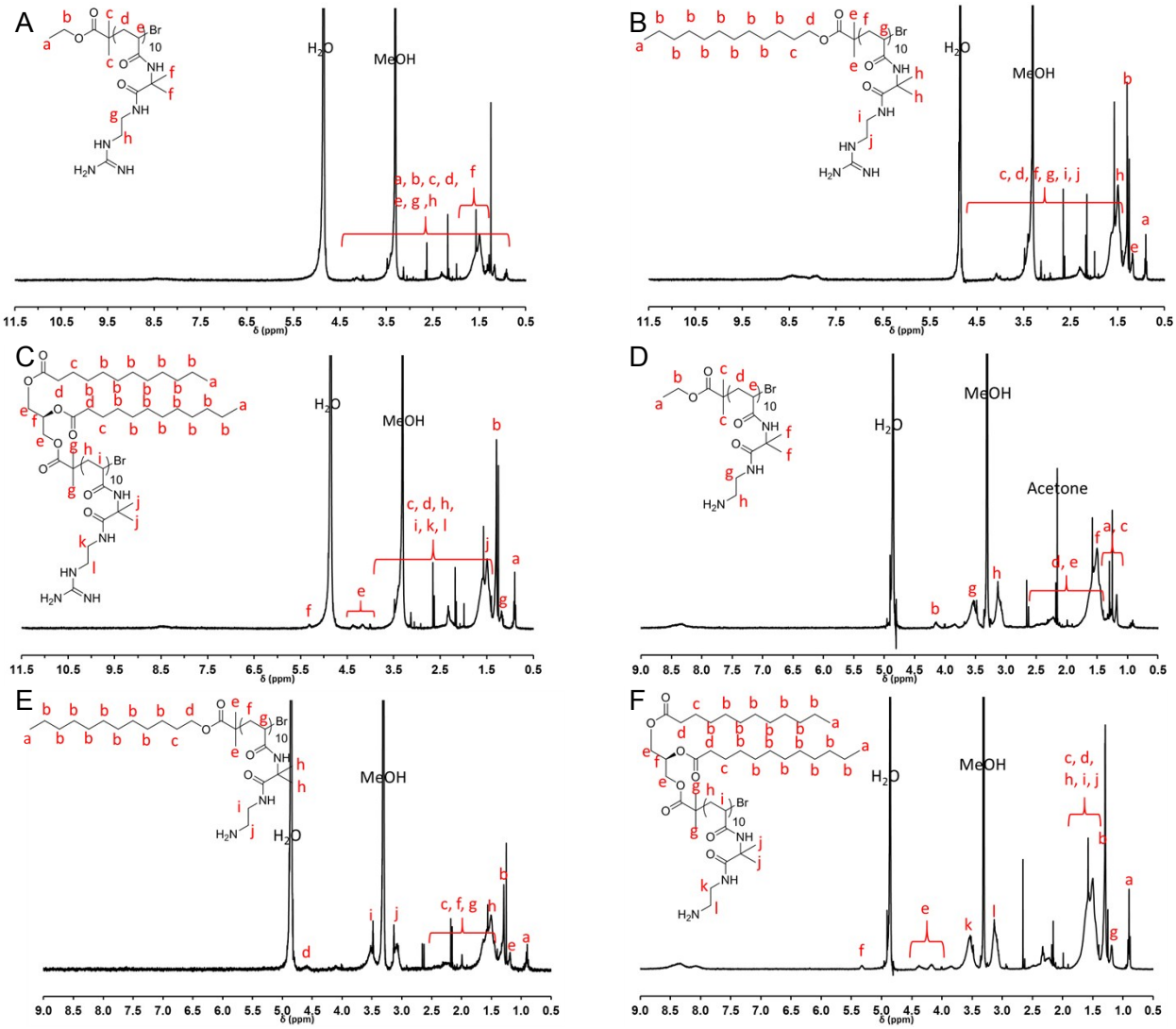


Figure SI 16 ^{1}H NMR spectra of deprotected oligomers; A) C_2-O(BG-D); B) C_{12}-O(BG-D); C) $2C_{12}$-O(BG-D); D) C_2-O(BEDA-D); E) C_{12}-O(BEDA-D); and F) $2C_{12}$-O(BEDA-D). ^{1}H NMR conducted in MeOD.

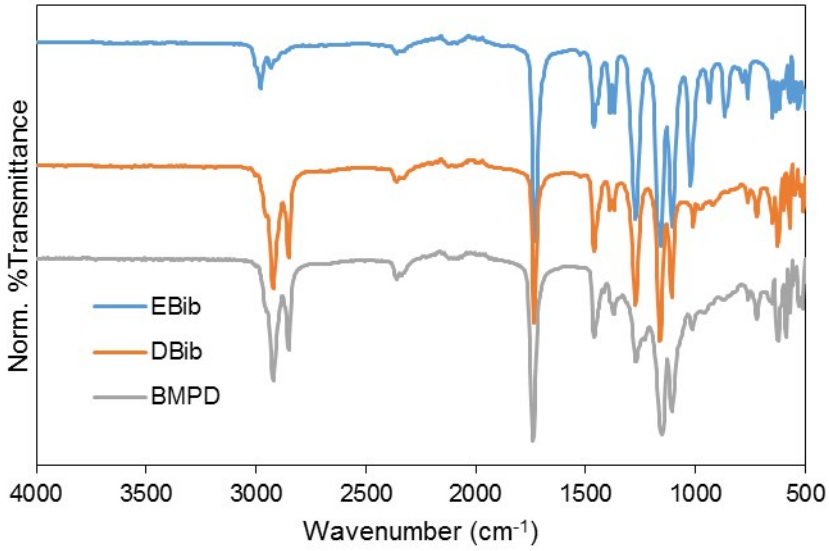


Figure SI 17 ATR-FTIR spectra for EBiB (blue); DBiB (orange); and BMPD (grey).

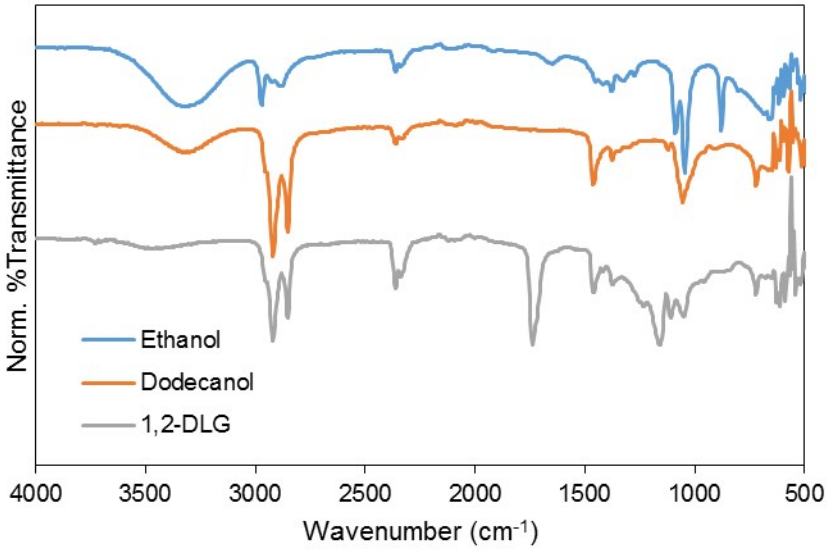


Figure SI 18 ATR-FTIR spectra of initiator precursors: ethanol (blue); dodecanol (orange); and 1,2-DLG (grey).

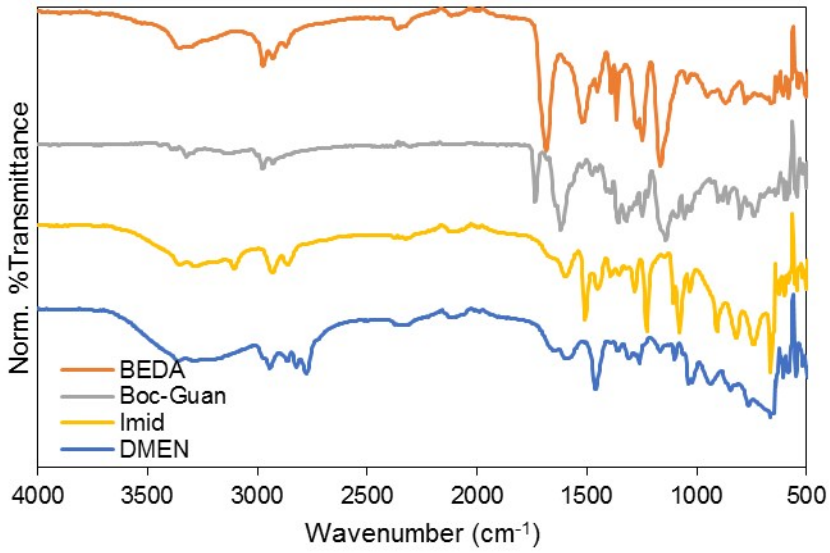


Figure SI 19 ATR-FTIR spectra of ring-opening reactant compounds: BEDA (orange); Boc-guanidine (grey); Imidazole (yellow); and DMEN (blue).

Table SI 1 Antibacterial testing of oligomer compounds against E. coli ATCC 25922 and methicillin-resistant S. aureus (MRSA) ATCC 43300 in CaMHB at pH 5.5 and 7.4.

Oligomer	*E. coli*		*S. aureus*	
	pH 5.5	pH 7.4	pH 5.5	pH 7.4
Polymyxin B	0.5 – 1	0.125 – 0.5	-	-
Vancomycin	-	-	1 – 2	1 – 2
C_2-O(DMEN)	512 – >512	>512	>512	>512
C_{12}-O(DMEN)	128 – 256	64 – 128	512 – >512	512
$2C_{12}$-O(DMEN)	256-512	>512	>512	>512
C_2-O(Imid)	>512	>512	>512	>512
C_{12}-O(Imid)	>512	>512	>512	>512
$2C_{12}$-O(Imid)	>512	>512	>512	>512
C_2-O(BEDA-D)	>512	>512	>512	512
C_{12}-O(BEDA-D)	128 – 256	128 – 256	512	16 – 32
$2C_{12}$-O(BEDA-D)	512 – >512	512	>512	128
C_2-O(BG-D)	>512	>512	>512	256
C_{12}-O(BG-D)	256	256	256	8 – 16
$2C_{12}$-O(BG-D)	512	256 – >512	256	64 – 128

Table SI 2 Cytotoxicity testing of oligomer compounds against human red bloods cells and HEK293 ATCC CRL-1573 cells.

Oligomer	Haemolysis		HEK293
	HC_{10} (μg/mL)	HC_{50} (μg/mL)	CC_{50} (μg/mL)
C_2-O(DMEN)	>217	>217	>256
C_{12}-O(DMEN)	>217	>217	117
$2C_{12}$-O(DMEN)	>217	>217	59

C_2-O(Imid)	>217	>217	>256
C_{12}-O(Imid)	>217	>217	69
$2C_{12}$-O(Imid)	>217	>217	>256
C_2-O(BEDA-D)	>217	>217	>256
C_{12}-O(BEDA-D)	43	>217	137
$2C_{12}$-O(BEDA-D)	10	>217	64
C_2-O(BG-D)	>217	>217	>256
C_{12}-O(BG-D)	38	>217	56
$2C_{12}$-O(BG-D)	7.1	108	66
Polymyxin B	>217	>217	>256
Colistin	>217	>217	>256
Vancomycin	>217	>217	>256
Tamoxifen			12 - 13
Melittin	3.2 - 3.8	8 - 8.8	

Table SI 3 Selectivity index values[a] for the oligomer library against E. coli and S. aureus at pH 5.5 and 7.4.[b]

Oligomer	HC_{10}[c] (μg mL^{-1})	*E. coli*		*S. aureus*	
		pH 5.5	pH 7.4	pH 5.5	pH 7.4
C_2-O(DMEN)	>217	ND[d]->0.42			
C_{12}-O(DMEN)	>217	>0.85-1.70	1.70-3.39	ND[d]->0.42	>0.42
$2C_{12}$-O(DMEN)	>217	>0.42-0.85			
C_2-O(Imid)	>217				
C_{12}-O(Imid)	>217				
$2C_{12}$-O(Imid)	>217				
C_2-O(BEDA-D)	>217				>0.42
C_{12}-O(BEDA-D)	43	0.17-0.34	0.17-0.34	0.08	1.34-2.69
$2C_{12}$-O(BEDA-D)	10	<0.02	0.02	<0.02	0.08
C_2-O(BG-D)	>217				>0.85
C_{12}-O(BG-D)	38	0.15	0.15	0.15	2.38-4.75
$2C_{12}$-O(BG-D)	7.1	0.014	>0.014-0.028	0.028	0.055-0.11

[a]The selectivity index is calculated by dividing the HC_{10} by the MIC value for a given microbe. The selectivity index is used routinely[1,2] to describe the selectivity of the compound toward a particular microbe. [b]MIC values are shown in Table SI 1. [c]HC_{10} values were calculated by interpolation of results in Figure 4. [c]ND stands for not determined, as the concentration for both MIC and HC_{10} were outside the range of concentration tested.

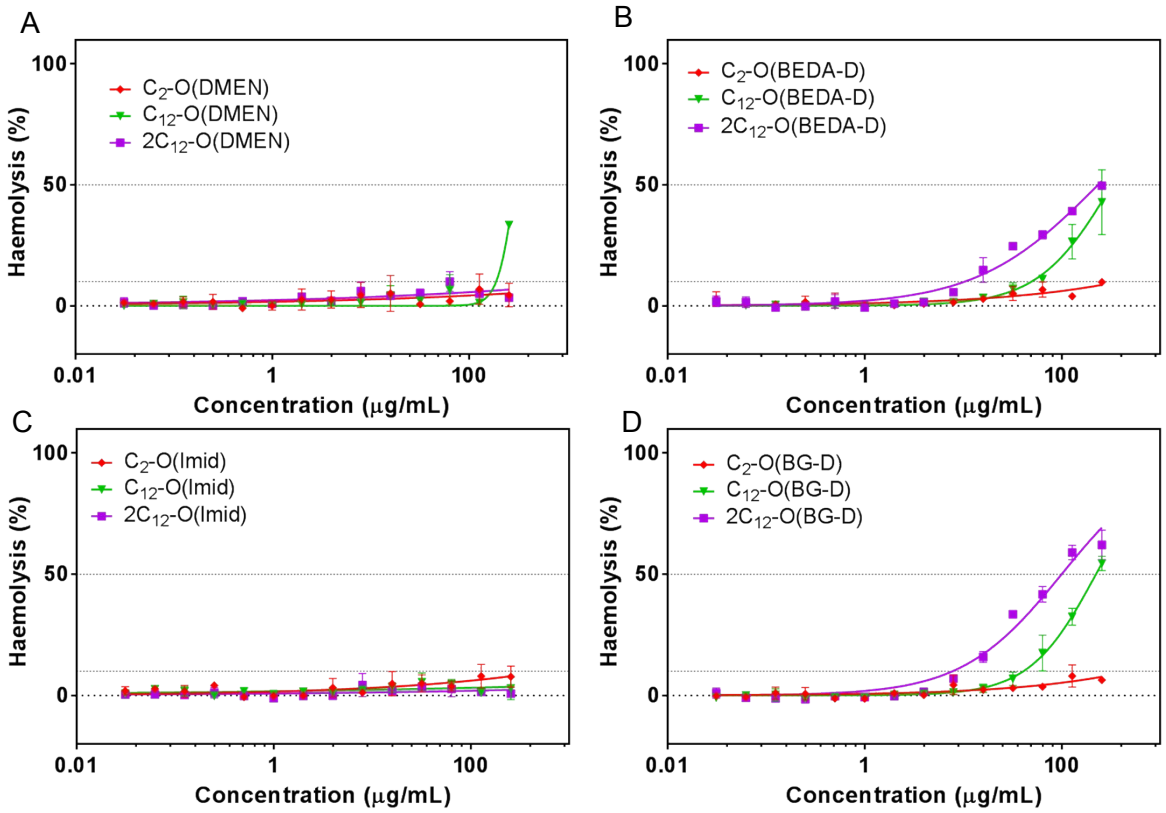


Figure SI 20 Haemolytic toxicity after 1 hour exposure to human red blood cells of oligomers containing: A) DMEN; B) BEDA-D; C) Imidazole; D) Boc-Guan-D. Data are presented as Mean ± Standard Deviation.

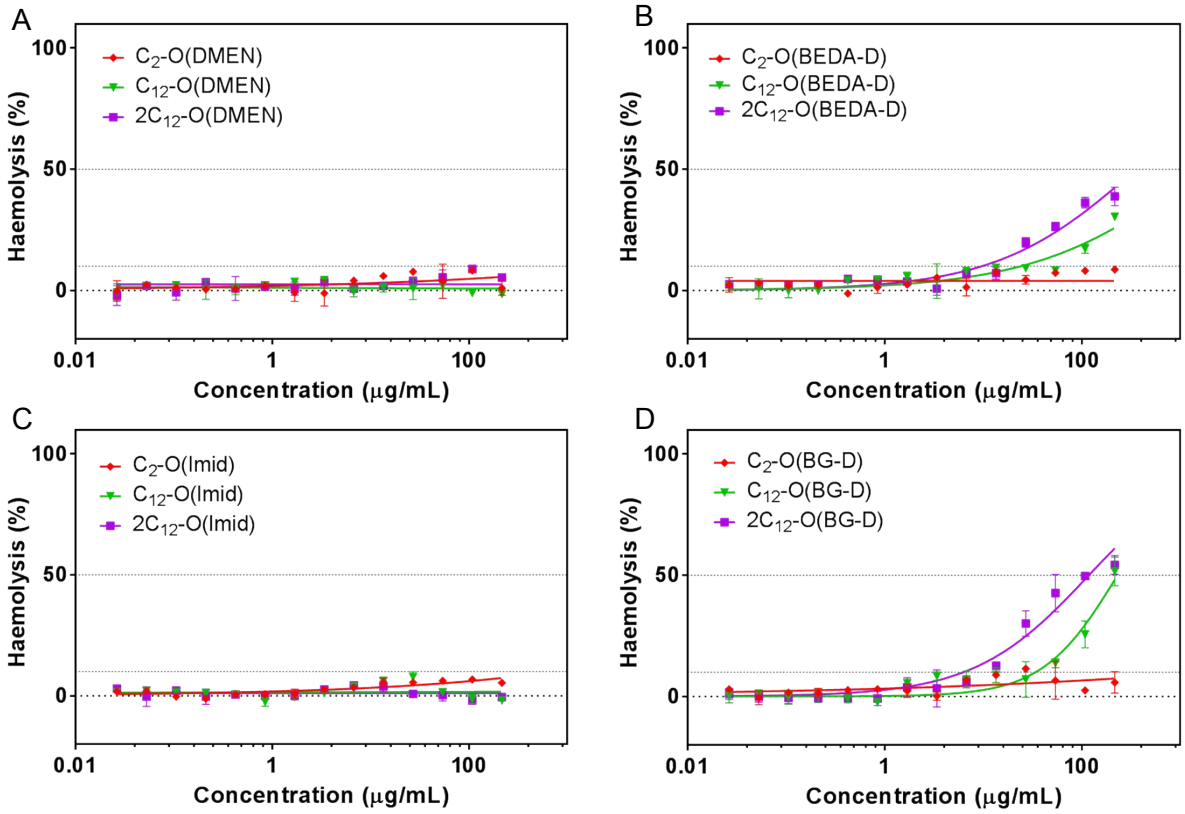


Figure SI 21 Haemolytic toxicity after 1 hour exposure to human red blood cells of oligomers containing: A) DMEN; B) BEDA-D; C) Imidazole; D) Boc-Guan-D. Data are presented as Mean ± Standard Deviation.

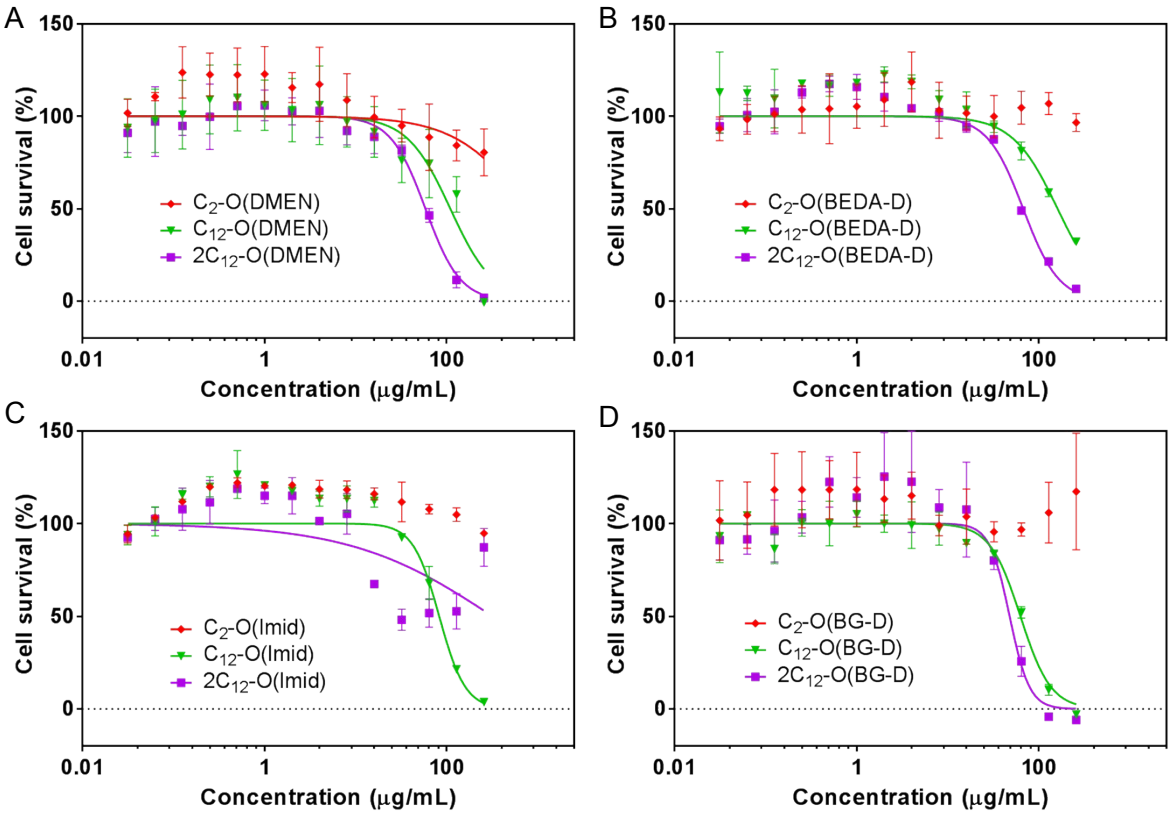


Figure SI 22 Cell viability vs concentration against HEK293 cells for oligomers containing: A) DMEN; B) BEDA-D; C) Imidazole; D) Boc-Guan-D. Data are presented as Mean ± Standard Deviation.

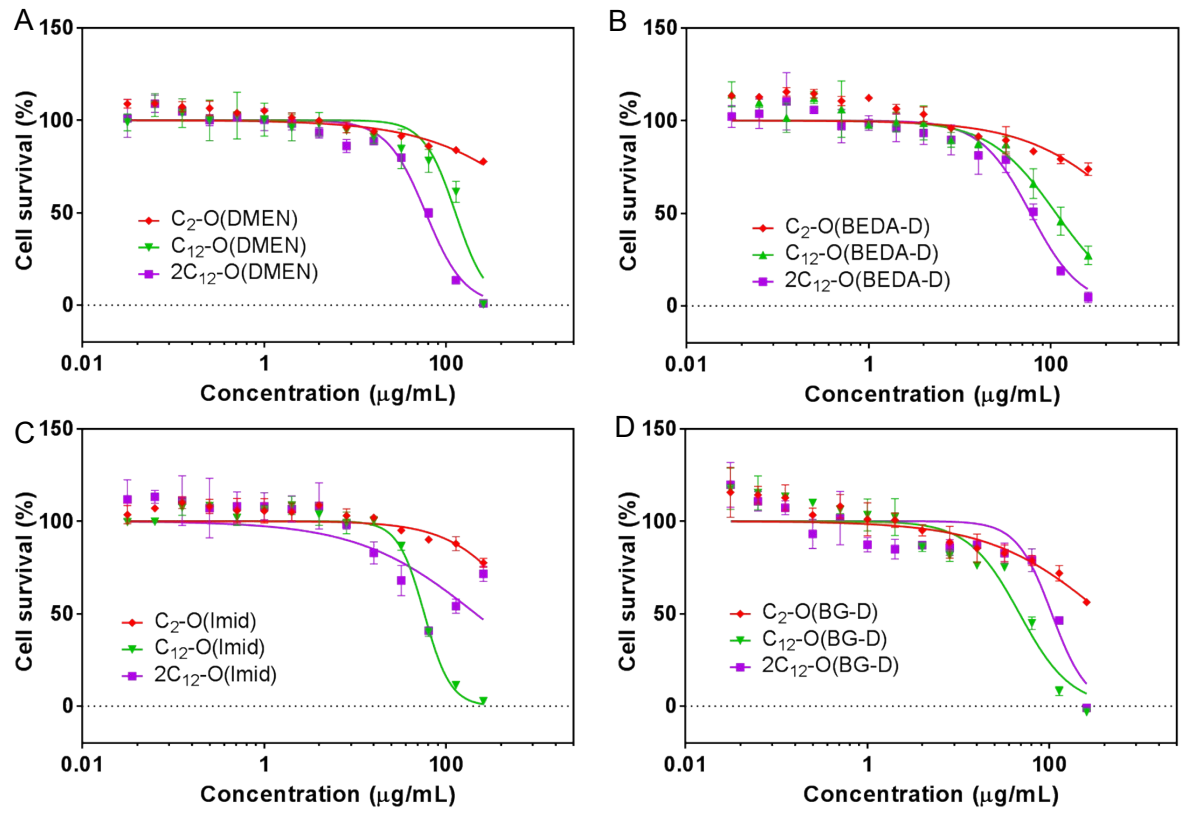


Figure SI 23 Cell viability vs concentration against HEK293 cells for oligomers containing: A) DMEN; B) BEDA-D; C) Imidazole; D) Boc-Guan-D. Data are presented as Mean ± Standard Deviation.

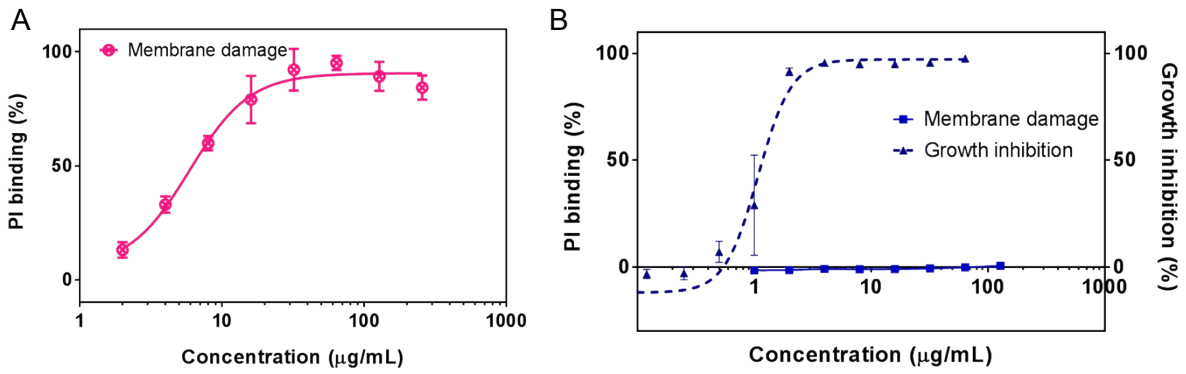


Figure SI 24 (A) PI binding (%) assay of the positive control: melittin; and (B) PI binding (%) assay and growth inhibition (%) assay of the negative control: Vancomycin over a concentration range. Data are presented as mean ± standard error of the mean (n=6).

Bibliography

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